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(54) Title: STABLE PHARMACEUTICAL COMPOSITION CONTAINING FACTOR VIII

(57) Abstract: The invention relates to a stable solid pharmaceutical composition comprising factor VIII. Such a composition is devoid of amino acids and comprises: (a) factor VIII; (b) a surfactant; (c) calcium chloride; (d) sucrose; (e) sodium chloride; (f) trisodium citrate; and (g) a buffer devoid of amino acids; and has a pH from 6 to 8 prior to lyophilisation and after reconstitution in water for injection. The invention also relates to the liquid pharmaceutical composition obtainable after dilution of said stable solid pharmaceutical composition with sterile water optionally containing sodium chloride.

Stable pharmaceutical composition containing factor VIII

The invention relates to a new stable pharmaceutical composition containing factor VIII.

Factor VIII is a well-known plasma protein that is essential to the blood clotting process and is therefore used in the treatment of haemophilia.

- 5 Several forms of factor VIII have been used or are intended to be used as active principles for treating haemophilia. These include human factor VIII (like the active principles of Humate® P, Monoclote® P, Immunate® or Hemofil® M), recombinant human factor VIII (like r-VIII SQ which is described in PCT patent application WO 91/09122 (the active principle of ReFacto®) or the active principles of Kogenate® or Recombinate®), porcine factor VIII (which is the active principle of the product Hyate:C® sold by Ipsen, Inc., USA) or recombinant porcine factor VIII (e.g. a modified B-domainless form of porcine factor VIII like the one disclosed in patent application WO 01/68109 and identified as "POL1212" or the protein of SEQ. ID. NO. 38 of the same patent application).
- 10
- 15 Formulation stability has always been a problem for the pharmaceutical industry dealing with factor VIII pharmaceutical compositions.

- 20
- Albumin has often been used to stabilise these formulations. However, despite its interesting stabilising effect, albumin presents the drawback of being expensive and also the risk to carry infectious species like prions. For these reasons, the pharmaceutical industry has been seeking in the recent years to replace albumin by other stabilising agents in factor VIII pharmaceutical compositions.

Several stable albumin-free pharmaceutical compositions are already known to the skilled artisan. For example:

- US patent No. 5,565,427 teaches a stabilised albumin-free solution with factor VIII:C activity containing factor VIII:C, an amino acid or one of its salts or homologues and a detergent (like polysorbate 80 or Tween® 80) or an organic polymer (like polyethyleneglycol).
- 25
- US patent No. 5,605,884 relates to a stable factor VIII composition comprising factor VIII and a high ionic strength media, which is preferably consisting of an aqueous

solution comprising a mixture of sodium chloride, calcium chloride and histidine as buffer ion.

- US patents Nos. 5,763,401 and 5,874,408 both disclose a stable albumin-free recombinant factor VIII composition comprising recombinant factor VIII, glycine, histidine, sucrose, sodium chloride and calcium chloride.

5 - US patent No. 5,962,650 teaches a stable albumin-free recombinant factor VIII composition which consists of an aqueous solution with a reduced concentration of oxygen comprising recombinant factor VIII, a calcium salt like calcium chloride and preferably an antioxidant, a non-ionic surfactant, sodium or potassium chloride, an 10 amino acid and a mono- or disaccharide.

15 - US patent No. 5,972,885 relates to a pharmaceutical formulation for subcutaneous, intramuscular or intradermal administration which comprises highly concentrated (at least 1,000 IU/ml) recombinant factor VIII and, preferably, one or more elements selected from the group constituted (notably) by sodium or potassium chloride, calcium chloride, a non-ionic surfactant (e.g. a poloxamer), a mono- or disaccharide (preferably sucrose) and antioxidants (e.g. citric acid).

20 - PCT patent application WO 89/09784 discloses a method for the production of heat-stable factor VIII concentrate which comprises gel filtration of a buffer solution containing said factor VIII and tris(hydroxymethyl)methylamine, trisodium citrate, sodium chloride, sucrose and calcium chloride followed by freeze-drying of the 25 concentrate obtained. The factor VIII thus produced is able to withstand temperatures of up to 80 °C for up to 72 hours.

25 - PCT patent application WO 94/07510 describes a factor VIII composition which is stabilised by a non-ionic surfactant (e.g. a poloxamer like polysorbate 80). Such a composition can also comprise one or more elements selected from the group constituted (notably) by sodium or potassium chloride, calcium chloride, an amino acid, a mono- or disaccharide such as sucrose,

30 The Applicant has now unexpectedly discovered that a solid pharmaceutical composition obtainable by lyophilisation of a solution devoid of amino acids comprising the following components:

(a) factor VIII;

(b) a surfactant;

- (c) calcium chloride;
- (d) sucrose;
- (e) sodium chloride;
- (f) trisodium citrate; and
- 5 (g) a buffer devoid of amino acids;

said pharmaceutical composition having a pH from 6 to 8 prior to lyophilisation and after reconstitution in water for injection, also shows stability over time.

10 By factor VIII is meant in the present application human factor VIII, recombinant human factor VIII, porcine factor VIII, recombinant porcine factor VIII or more generally any other recombinant factor VIII that can be used to replace them.

15 Preferably, the factor VIII comprised in compositions according to the invention, will be chosen from porcine factor VIII or recombinant porcine factor VIII. Still more preferably, the factor VIII comprised in compositions according to the invention, will be recombinant porcine factor VIII, especially a modified B-domainless form of porcine factor VIII like the one disclosed in patent application WO 01/68109, i.e. the modified porcine factor VIII having the amino acid sequence SEQ. ID. NO. 1 hereafter:

SEQ. ID. NO. 1:

Met	Gln	Leu	Glu	Leu	Ser	Thr	Cys	Val	Phe	Leu	Cys	Leu	Leu	Pro	Leu
1				5					10					15	
Gly	Phe	Ser	Ala	Ile	Arg	Arg	Tyr	Tyr	Leu	Gly	Ala	Val	Glu	Leu	Ser
				20				25					30		
Trp	Asp	Tyr	Arg	Gln	Ser	Glu	Leu	Leu	Arg	Glu	Leu	His	Val	Asp	Thr
				35			40					45			
Arg	Phe	Pro	Ala	Thr	Ala	Pro	Gly	Ala	Leu	Pro	Leu	Gly	Pro	Ser	Val
				50			55				60				
Leu	Tyr	Lys	Lys	Thr	Val	Phe	Val	Glu	Phe	Thr	Asp	Gln	Leu	Phe	Ser
				65		70			75				80		
Val	Ala	Arg	Pro	Arg	Pro	Pro	Trp	Met	Gly	Leu	Leu	Gly	Pro	Thr	Ile
				85				90					95		
Gln	Ala	Glu	Val	Tyr	Asp	Thr	Val	Val	Val	Thr	Leu	Lys	Asn	Met	Ala
				100				105					110		
Ser	His	Pro	Val	Ser	Leu	His	Ala	Val	Gly	Val	Ser	Phe	Trp	Lys	Ser
				115			120					125			
Ser	Glu	Gly	Ala	Glu	Tyr	Glu	Asp	His	Thr	Ser	Gln	Arg	Glu	Lys	Glu
				130			135					140			

Asp Asp Lys Val Leu Pro Gly Lys Ser Gln Thr Tyr Val Trp Gln Val
 145 150 155 160
 Leu Lys Glu Asn Gly Pro Thr Ala Ser Asp Pro Pro Cys Leu Thr Tyr
 165 170 175
 Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu
 180 185 190
 Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Thr Arg Glu Arg
 195 200 205
 Thr Gln Asn Leu His Glu Phe Val Leu Leu Phe Ala Val Phe Asp Glu
 210 215 220
 Gly Lys Ser Trp His Ser Ala Arg Asn Asp Ser Trp Thr Arg Ala Met
 225 230 235 240
 Asp Pro Ala Pro Ala Arg Ala Gln Pro Ala Met His Thr Val Asn Gly
 245 250 255
 Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Lys Lys Ser
 260 265 270
 Val Tyr Trp His Val Ile Gly Met Gly Thr Ser Pro Glu Val His Ser
 275 280 285
 Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg His His Arg Gln Ala
 290 295 300
 Ser Leu Glu Ile Ser Pro Leu Thr Phe Leu Thr Ala Gln Thr Phe Leu
 305 310 315 320
 Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His His
 325 330 335
 His Gly Gly Met Glu Ala His Val Arg Val Glu Ser Cys Ala Glu Glu
 340 345 350
 Pro Gln Leu Arg Arg Lys Ala Asp Glu Glu Asp Tyr Asp Asp Asn
 355 360 365
 Leu Tyr Asp Ser Asp Met Asp Val Val Arg Leu Asp Gly Asp Asp Val
 370 375 380
 Ser Pro Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
 385 390 395 400
 Trp Val His Tyr Ile Ser Ala Glu Glu Asp Trp Asp Tyr Ala Pro
 405 410 415
 Ala Val Pro Ser Pro Ser Asp Arg Ser Tyr Lys Ser Leu Tyr Leu Asn
 420 425 430
 Ser Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Ala Arg Phe Val
 435 440 445
 Ala Tyr Thr Asp Val Thr Phe Lys Thr Arg Lys Ala Ile Pro Tyr Glu
 450 455 460
 Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
 465 470 475 480
 Leu Ile Ile Phe Lys Asn Lys Ala Ser Arg Pro Tyr Asn Ile Tyr Pro

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485	490	495
His Gly Ile Thr Asp Val Ser Ala Leu His Pro Gly Arg Leu Leu Lys		
500	505	510
Gly Trp Lys His Leu Lys Asp Met Pro Ile Leu Pro Gly Glu Thr Phe		
515	520	525
Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp		
530	535	540
Pro Arg Cys Leu Thr Arg Tyr Ser Ser Ser Ile Asn Leu Glu Lys		
545	550	555
Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu		
565	570	575
Ser Val Asp Gln Arg Gly Asn Gln Met Met Ser Asp Lys Arg Asn Val		
580	585	590
Ile Leu Phe Ser Val Phe Asp Glu Asn Gln Ser Trp Tyr Leu Ala Glu		
595	600	605
Asn Ile Gln Arg Phe Leu Pro Asn Pro Asp Gly Leu Gln Pro Gln Asp		
610	615	620
Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val		
625	630	635
Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp		
645	650	655
Tyr Ile Leu Ser Val Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe		
660	665	670
Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr		
675	680	685
Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro		
690	695	700
Gly Leu Trp Val Leu Gly Cys His Asn Ser Asp Leu Arg Asn Arg Gly		
705	710	715
Met Thr Ala Leu Leu Lys Val Tyr Ser Cys Asp Arg Asp Ile Gly Asp		
725	730	735
Tyr Tyr Asp Asn Thr Tyr Glu Asp Ile Pro Gly Phe Leu Leu Ser Gly		
740	745	750
Lys Asn Val Ile Glu Pro Arg Ser Phe Ala Gln Asn Ser Arg Pro Pro		
755	760	765
Ser Ala Ser Ala Pro Lys Pro Pro Val Leu Arg Arg His Gln Arg Asp		
770	775	780
Ile Ser Leu Pro Thr Phe Gln Pro Glu Glu Asp Lys Met Asp Tyr Asp		
785	790	795
Asp Ile Phe Ser Thr Glu Thr Lys Gly Glu Asp Phe Asp Ile Tyr Gly		
805	810	815
Glu Asp Glu Asn Gln Asp Pro Arg Ser Phe Gln Lys Arg Thr Arg His		
820	825	830

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Tyr Phe Ile Ala Ala Val Glu Gln Leu Trp Asp Tyr Gly Met Ser Glu
 835 840 845
 Ser Pro Arg Ala Leu Arg Asn Arg Ala Gln Asn Gly Glu Val Pro Arg
 850 855 860
 Phe Lys Lys Val Val Phe Arg Glu Phe Ala Asp Gly Ser Phe Thr Gln
 865 870 875 880
 Pro Ser Tyr Arg Gly Glu Leu Asn Lys His Leu Gly Leu Leu Gly Pro
 885 890 895
 Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Lys Asn
 900 905 910
 Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Pro
 915 920 925
 Asp Asp Gln Glu Gln Gly Ala Glu Pro Arg His Asn Phe Val Gln Pro
 930 935 940
 Asn Glu Thr Arg Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro
 945 950 955 960
 Thr Glu Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val
 965 970 975
 Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Ile
 980 985 990
 Cys Arg Ala Asn Thr Leu Asn Ala Ala His Gly Arg Gln Val Thr Val
 995 1000 1005
 Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp
 1010 1015 1020
 Tyr Phe Thr Glu Asn Val Glu Arg Asn Cys Arg Ala Pro Cys His Leu
 1025 1030 1035 1040
 Gln Met Glu Asp Pro Thr Leu Lys Glu Asn Tyr Arg Phe His Ala Ile
 1045 1050 1055
 Asn Gly Tyr Val Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asn
 1060 1065 1070
 Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile
 1075 1080 1085
 His Ser Ile His Phe Ser Gly His Val Phe Ser Val Arg Lys Lys Glu
 1090 1095 1100
 Glu Tyr Lys Met Ala Val Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr
 1105 1110 1115 1120
 Val Glu Met Leu Pro Ser Lys Val Gly Ile Trp Arg Ile Glu Cys Leu
 1125 1130 1135
 Ile Gly Glu His Leu Gln Ala Gly Met Ser Thr Thr Phe Leu Val Tyr
 1140 1145 1150
 Ser Lys Glu Cys Gln Ala Pro Leu Gly Met Ala Ser Gly Arg Ile Arg
 1155 1160 1165
 Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys
 1170 1175 1180

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Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys
 1185 1190 1195 1200
 Asp Pro His Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile
 1205 1210 1215
 His Gly Ile Met Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr
 1220 1225 1230
 Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Arg Asn Trp Gln
 1235 1240 1245
 Ser Arg Glu Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn
 1250 1255 1260
 Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Val
 1270 1275 1280
 Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr
 1285 1290 1295
 Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro
 1300 1305 1310
 Leu Gly Met Gln Asn Lys Ala Ile Ser Asp Ser Gln Ile Thr Ala Ser
 1315 1320 1325
 Ser His Leu Ser Asn Ile Phe Ala Thr Trp Ser Pro Ser Gln Ala Arg
 1330 1335 1340
 Leu His Leu Gln Gly Arg Thr Asn Ala Trp Arg Pro Arg Val Ser Ser
 1345 1350 1355 1360
 Ala Glu Glu Trp Leu Gln Val Asp Leu Gln Lys Thr Val Lys Val Thr
 1365 1370 1375
 Gly Ile Thr Thr Gln Gly Val Lys Ser Leu Leu Ser Ser Met Tyr Val
 1380 1385 1390
 Lys Glu Phe Leu Val Ser Ser Gln Asp Gly Arg Arg Trp Thr Leu
 1395 1400 1405
 Phe Leu Gln Asp Gly His Thr Lys Val Phe Gln Gly Asn Gln Asp Ser
 1410 1415 1420
 Ser Thr Pro Val Val Asn Ala Leu Asp Pro Pro Leu Phe Thr Arg Tyr
 1425 1430 1435 1440
 Leu Arg Ile His Pro Thr Ser Trp Ala Gln His Ile Ala Leu Arg Leu
 1445 1450 1455
 Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr
 1460 1465

Preferably, the surfactant will be a non-ionic surfactant. Non-ionic surfactants include notably polysorbates and block copolymers like poloxamers (i.e. copolymers of polyethylene and propylene glycol). According to a preferred variant of the invention, the surfactant will be a polysorbate. More preferably, a polysorbate included in a composition according to the instant invention will have a mean polymerisation degree

of from 20 to 100 monomer units (preferably about 80), and may for example be polysorbate 80. Preferably also, the polysorbate should be vegetable-derived.

Preferably, the buffer devoid of amino acids will be tris(hydroxymethyl)methylamine (hereafter abridged "tris").

5 Preferably also, the pH of the pharmaceutical composition prior to lyophilisation and after reconstitution in water for injection will be from 6.5 to 7.5, and more preferably about 7.0.

Preferably, a solid composition according to the invention will be such that it may be obtained by lyophilisation of a solution devoid of amino acids that comprises:

10 (a) a concentration of factor VIII ranging from 50 to 10,000 international units/ml for human or recombinant human factor VIII or from 50 to 10,000 porcine units/ml for porcine or recombinant porcine factor VIII;

(b) a concentration of surfactant ranging from above critical micellar concentration to 1% v/v;

15 (c) a concentration of calcium chloride ranging from 0.5 to 10 mM;

(d) a concentration of sucrose ranging from 5 to 50 mM;

(e) a concentration of sodium chloride ranging from 0.15 to 0.5 M;

(f) a concentration of trisodium citrate ranging from 1 to 50 mM; and

(g) a concentration of buffer devoid of amino acids ranging from 1 to 50 mM.

20 For evaluating the activity in terms of international factor VIII units, the product to be tested is assayed against a Concentrate Standard, such as the United Kingdom standard NIBSC 95/608 (NIBSC for National Institute of Biological Standards and Control).

By porcine unit of factor VIII is meant the United Kingdom national standard unit held by United Kingdom's NIBSC. For evaluating the activity in terms of porcine factor VIII

25 units, the product to be tested is assayed against the UK national porcine standard NIBSC 86/514. Concerning recombinant porcine factor VIII, it should be understood that 1 unit of activity for recombinant porcine factor VIII is equivalent to 1 unit of activity for porcine factor VIII.

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More preferably, a solid composition according to the invention will be such that it may be obtained by lyophilisation of a solution devoid of amino acids that comprises at least one of the following characteristics:

- 5 • a concentration of factor VIII ranging from 100 to 5,000 international units/ml for human or recombinant human factor VIII or from 100 to 5,000 porcine units/ml for porcine or recombinant porcine factor VIII;
- 10 • a concentration of surfactant ranging from 0.002% to 0.04% v/v;
- a concentration of calcium chloride ranging from 1 to 5 mM;
- a concentration of sucrose ranging from 5 to 25 mM;
- 15 • a concentration of sodium chloride ranging from 0.2 to 0.4 M;
- a concentration of trisodium citrate ranging from 1 to 20 mM; or
- a concentration of buffer devoid of amino acids ranging from 1 to 20 mM.

Even more preferably, a solid composition according to the invention will be such that it may be obtained by lyophilisation of a solution devoid of amino acids that comprises at least one of the following characteristics:

- 20 • a concentration of factor VIII ranging from 200 to 2,000 international units/ml (and notably about 1,000 international units/ml) for human or recombinant human factor VIII or from 200 to 2,000 porcine units/ml (and notably about 1,000 porcine units/ml) for porcine or recombinant porcine factor VIII;
- a concentration of surfactant ranging from about 0.002% to 0.02% v/v (and notably about 0.01% v/v);
- a concentration of calcium chloride ranging from 1 to 3 mM (and notably about 2 mM);
- a concentration of sucrose ranging from 5 to 15 mM (and notably about 11.7 mM);
- 25 • a concentration of sodium chloride ranging from 0.25 to 0.35 M (and notably about 0.3 M);
- a concentration of trisodium citrate ranging from 1 to 20 mM (and notably about 10 mM); or

- a concentration of buffer devoid of amino acids ranging from 5 to 15 mM (and notably about 10 mM).

5 The solid factor VIII compositions according to the invention may be prepared by lyophilising a solution comprising the appropriate quantities of the components identified above as (a), (b), (c), (d), (e), (f) and (g) according to standard manufacturing procedures (sterile conditions, etc.).

10 Stability of the composition over a certain period may be determined, for example, by the method described hereunder in the part entitled "Analytical methods", or by any other method found appropriate by the skilled artisan.

15 10 A composition according to the invention is considered stable during a certain period of time if 70% to 130% (and preferably 80% to 120%) of the initial factor VIII activity, as evaluated using the method disclosed the part entitled "*Analytical methods*" hereafter, is maintained over said period of time.

20 15 Preferably, the solid compositions of this invention will be stable for at least 6 or 12 months when kept at a temperature of 2 to 8 °C. More preferably, they will be stable for at least 6 or 12 months when kept at a temperature of 30 to 32 °C.

25 The solid factor VIII compositions according to the invention may be diluted with sterile water optionally containing sodium chloride, and the resulting liquid pharmaceutical composition may then be directly injected into a patient in need thereof. 20 The resulting liquid pharmaceutical composition, as well as liquid pharmaceutical compositions obtainable by dilution of solid factor VIII compositions according to the invention with sterile water optionally containing sodium chloride, are also part of this invention.

30 25 Methods of treatment of haemophilia comprising the administration of a liquid composition according to the invention to a patient in need thereof are also within the scope of this invention. The administration mode contemplated for liquid compositions according to the instant invention will preferably be intravenous administration. The dose of composition according to the instant invention which is to be administered will be determined by the treating physician or veterinarian, taking into account the severity of the disease for each patient.

35 The term "about" refers to an interval around the considered value. As used in this patent application, "about X" means an interval from X minus 10% of X to X plus 10% of X, and preferably an interval from X minus 5% of X to X plus 5% of X.

Unless they are defined differently, all the technical and scientific terms used here have the same meaning as that usually understood by an ordinary specialist in the field to which this invention belongs. Similarly, all publications, patent applications, all patents and all other references mentioned here are incorporated by way of reference.

- 5 The following examples are presented to illustrate the above and must in no case be considered as a limit to the scope of the invention.

EXAMPLES

Example 1:

A solution in 0.5 ml sterile water containing the following components is prepared:

Modified porcine factor VIII of sequence SEQ. ID. NO. 1	800 porcine units/ml
Vegetable derived polysorbate 80	0.01% v/v
Calcium chloride	2 mM
Sucrose	11.7 mM
Sodium chloride	0.3 M
Tri sodium citrate	10 mM
Tris buffer	10 mM
pH	7.0

- 10 The mixture is lyophilised in a sterilised vial which is then sealed. The solid composition obtained has been tested and shown to be stable at a temperature of 2 to 8 °C for at least 18 months and at 30 to 32°C for at least six months when tested by factor VIII activity. There was no indication of high molecular weight component formation as assessed by Size Exclusion HPLC (SEC HPLC) or fragments as assessed by SDS PAGE.
- 15

The lyophilised mixture obtained would typically be reconstituted with 1.0 ml sterile water before injection into a patient.

Example 2:

A solution in 1.0 ml sterile water containing the following components is prepared:

Modified porcine factor VIII of sequence SEQ. ID. NO. 1	400 porcine units/ml
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Vegetable derived polysorbate 80	0.002% v/v
Calcium chloride	2 mM
Sucrose	11.7 mM
Sodium chloride	0.3 M
Tri sodium citrate	10 mM
Tris buffer	10 mM
pH	7.0

The mixture is lyophilised in a sterilised vial which is then sealed.

The lyophilised mixture obtained would typically be reconstituted with 2.0 ml sterile water before injection into a patient.

Example 3:

5 A solution in 0.5 ml sterile water containing the following components is prepared:

Plasma-derived porcine factor VIII	100 porcine units/ml
Vegetable derived polysorbate 80	0.01% v/v
Calcium chloride	2 mM
Sucrose	11.7 mM
Sodium chloride	0.3 M
Tri sodium citrate	10 mM
Tris buffer	10 mM
pH	7.0

The mixture is lyophilised in a sterilised vial which is then sealed.

The lyophilised mixture obtained would typically be reconstituted with 1.0 ml sterile water before injection into a patient.

ANALYTICAL METHODS

10 *Chromogenic assay*

The factor VIII activity is determined by a modified chromogenic assay (Technochrom FVIII:C Reagent Kit, Technoclone). The generation of activated factor X by factor IX is stimulated by factor VIII which acts as a cofactor in the reaction. The release of

p-nitroaniline from the chromogenic substrate is catalysed by activated factor X. The amount of *p*-nitroaniline which is released is measured photometrically at 405 nm and the assay gives a linear correlation between the amount of *p*-nitroaniline generated and the FVIII content.

5 *SEC HPLC*

Soluble high molecular weight components and fragments were determined by gel filtration performed on a HPLC instrument using a TosoHaas TSK G3000 SWXL, 0.78 x 30 cm pre-packed column with a fluorescence detector (Waters LC Module 1 plus). Excitation wavelength 280 nm and emission wavelength 340 nm. Evaluation of the 10 results were performed by integration of the peak areas.

SDS PAGE assay

SDS PAGE (polyacrylamide gel electrophoresis using a flatbed electrophoresis system (Multiphor II LKB) and pre cast 7.5% gels (EXCELGEL SDS, Pharmacia) was used to determine any breakdown products of the FVIII molecule. Protein bands were visualised 15 by Coomassie blue staining.

Stability assay

Stability can be assayed by performing the above described assays at different times on a sample of the same composition held at the temperature chosen (which may be around + 4 °C or + 31 °C). Once its factor VIII activity will have dropped of more than 30%, 20 the composition will be considered to have lost its stability.

CLAIMS

1. A solid pharmaceutical composition obtainable by lyophilisation of a solution devoid of amino acids comprising

- (a) factor VIII ;
- 5 (b) a surfactant;
- (c) calcium chloride;
- (d) sucrose;
- (e) sodium chloride;
- (f) trisodium citrate; and
- 10 (g) a buffer devoid of amino acids;

having a pH from 6 to 8 prior to lyophilisation and after reconstitution in water for injection.

2. A solid pharmaceutical composition according to claim 1 characterised in that the factor VIII is chosen from porcine factor VIII or recombinant porcine factor VIII.

15 3. A solid pharmaceutical composition according to claim 2 characterised in that the factor VIII is recombinant porcine factor VIII.

4. A solid pharmaceutical composition according to claim 2 characterised in that the recombinant porcine factor VIII has the amino acid sequence SEQ. ID. NO. 1.

20 5. A solid pharmaceutical composition according to one of claims 1 to 4 characterised in that the surfactant is a polysorbate.

6. A solid pharmaceutical composition according to claim 5 characterised in that the surfactant is a polysorbate 80.

7. A solid pharmaceutical composition according to one of claims 1 to 6 characterised in that the buffer devoid of amino acids is tris(hydroxymethyl)methylamine.

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8. A solid pharmaceutical composition according to one of claims 1 to 7 which, prior to lyophilisation and after reconstitution in water for injection, has a pH from 6.5 to 7.5.
9. A solid pharmaceutical composition according to one of claims 1 to 8, which may be obtained by lyophilisation of a solution devoid of amino acids that comprises:
 - 5 (a) a concentration of factor VIII ranging from 50 to 10,000 international units/ml for human or recombinant human factor VIII or from 50 to 10,000 porcine units/ml for porcine or recombinant porcine factor VIII;
 - (b) a concentration of surfactant ranging from above critical micellar concentration to 1% v/v;
 - 10 (c) a concentration of calcium chloride ranging from 0.5 to 10 mM;
 - (d) a concentration of sucrose ranging from 5 to 50 mM;
 - (e) a concentration of sodium chloride ranging from 0.15 to 0.5 M;
 - (f) a concentration of trisodium citrate ranging from 1 to 50 mM; and
 - (g) a concentration of a buffer devoid of amino acids ranging from 1 to 50 mM.
- 15 10. A liquid pharmaceutical composition obtainable after dilution of a solid pharmaceutical composition according to one of claims 1 to 9 with sterile water optionally containing sodium chloride.

44284 Ep.txt
SEQUENCE LISTING

<110> Société de Conseils de Recherches et d'Applications Scientifiques
(S.C.R.A.S.)

<120> stable pharmaceutical composition containing factor VIII

<130> 44284.W001/JMD

<150> GB 0207092.8

<151> 2003-03-26

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<211> 1467

<212> PRT

<213> Porcine

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44284 Ep.txt
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305 310 315 320Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His His
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44284 Ep.txt
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44284 Ep.txt
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Tyr Phe Ile Ala Ala Val Glu Gln Leu Trp Asp Tyr Gly Met Ser Glu
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Phe Lys Lys Val Val Phe Arg Glu Phe Ala Asp Gly Ser Phe Thr Gln
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Pro Ser Tyr Arg Gly Glu Leu Asn Lys His Leu Gly Leu Leu Gly Pro
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Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Pro

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INTERNATIONAL SEARCH REPORT

PCT/GB 03/01297

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K38/37 A61K9/19 A61K47/26 A61K47/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1997</p> <p>OSTERBERG THOMAS ET AL: "Development of a freeze-dried albumin-free formulation of recombinant factor VIII SQ." Database accession no. PREV199799711323 XP009011546 abstract & PHARMACEUTICAL RESEARCH (NEW YORK), vol. 14, no. 7, 1997, pages 892-898, ISSN: 0724-8741 page 896, column 1, line 33-36 page 896, column 2, line 1-4</p> <p>—</p> <p style="text-align: center;">-/-</p>	1-10

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority, claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *Z* document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 June 2003	09/07/2003
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax. 31 651 epo nl Fax: (+31-70) 340-3016	Authorized officer Allnutt, S

INTERNATIONAL SEARCH REPORT

PCT/GB 03/01297

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 00 48635 A (BESMAN MARC ;BJORNSON ERIK (US); PIKAL MICHAEL (US); CARPENTER JOH) 24 August 2000 (2000-08-24) page 4, line 21,22 page 24, line 10,11 see formulation 11 in table 8 on pg 25 and stability data in table 10 on pg 28 example 6	1-10
A	WO 01 03726 A (MIKAELSSON MARIANNE ;PHARMACIA & UPJOHN AB (SE); SANDBERG HELENA () 18 January 2001 (2001-01-18) claims 1,9,14-16,22	1-10

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 0048635	A 24-08-2000	AU	2884300 A	04-09-2000
		BR	0008405 A	30-04-2002
		CA	2362927 A1	24-08-2000
		CN	1399560 T	26-02-2003
		CZ	20012996 A3	13-03-2002
		EP	1154796 A1	21-11-2001
		WO	0048635 A1	24-08-2000
WO 0103726	A 18-01-2001	AU	6193300 A	30-01-2001
		CA	2378751 A1	18-01-2001
		EP	1194161 A1	10-04-2002
		JP	2003504345 T	04-02-2003
		NO	20020120 A	28-02-2002
		WO	0103726 A1	18-01-2001